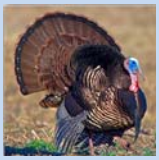


Detection of low pathogenic influenza viruses in eggs from infected turkeys

S.P.S. Pillai and Chang-Won Lee
Food Animal Health Research Program, Ohio Agricultural Research and Development Center,
The Ohio State University, Wooster, Ohio 44691

ABSTRACT

Studies on the potential of influenza viruses for egg borne transmission are limited. Experimental infection of breeder turkeys with H3N2 subtype influenza virus, A/turkey/Ohio/04, resulted in drastic declines in egg production and we confirmed high levels of virus replication and abundant distribution of avian type influenza receptors in the oviduct of turkeys. We also confirmed the presence of the virus in albumin of eggs using real time RT-PCR (RRT-PCR) and virus isolation in embryonated chicken eggs. Swabs from egg shells were also found positive by RRT-PCR. This is the first experimental demonstration of the transmission of low pathogenic avian influenza viruses to internal egg contents. Although there is no available evidence for vertical transmission of influenza viruses, the possibility of hatchery contamination as well as possible spread of virus during movement of contaminated cracked eggs as well as egg flats could be of serious food safety and public health concern.

INTRODUCTION

Although avian influenza viruses were detected from internal egg contents with high pathogenic isolates (1, 2), there are no reports on the egg borne transmission of low pathogenic isolates. Previously, we identified preferential tropism of H3N2 subtype influenza viruses for the oviduct of turkeys (3). In this study, we further investigated the possibility of eggs being contaminated with influenza viruses before or after oviposition and hence, internal or external contamination of eggs laid by infected turkeys.

OBJECTIVE

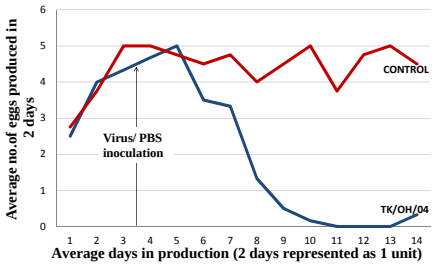
To determine whether viruses can be present in internal contents of eggs laid by breeder turkeys infected with low pathogenic influenza viruses.

METHODS

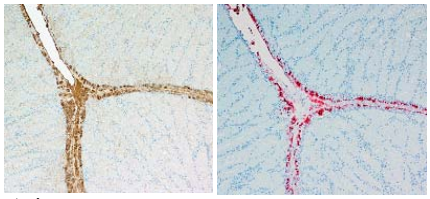
- Experimental infection of breeder turkeys with $10^{6.5}$ 50% tissue culture infectious dose ($TCID_{50}$) of turkey H3N2 virus, A/turkey/Ohio/04 (TK/OH/04), through choanal route.
- Determination of viral titers in the oviduct using RRT-PCR and detection of receptors and viral antigen in the oviduct using immunohistochemistry.
- Detection of viruses in homogenized albumin and egg shell swabs using RRT-PCR and virus isolation in embryonated eggs.

RESULTS

1. TK/OH/04 virus causes drastic declines in egg production in breeder turkeys



2. TK/OH/04 virus utilizes avian type receptors in the oviduct for binding



Avian – type receptors Viral antigen

Duplicate sections of isthmus showing exact match between avian receptors & viral antigen

3. TK/OH/04 virus replicates to high titers in different parts of the oviduct

Oviduct section	Viral titer*
Infundibulum	3.76
Magnum	5.84
Isthmus	4.96
Uterus	5.23

* Expressed as 50% egg infectious dose (EID_{50})/g of tissue

4. Detection of TK/OH/04 virus in albumin and egg shell surfaces

DPI[a]	Virus detection using RRT-PCR *			
	Albumin		Egg shell swab	
	Pos/total[b]	Viral titer[c]	Pos/total	Viral titer
2	5/22	2.30	8/22	4.62
3	3/15	2.35	3/12	2.55
4	4/12	3.13	7/11	1.89
5	1/10	2.94	10/10	3.73
6	1/3	1.52	1/3	1.60
7	0	0	0	0

[a]: Days post infection, [b]: No. positive/total eggs tested, [c]: Expressed as EID_{50}/100µl of albumin or PBS in which shell surface swab was suspended. * Selected RRT-PCR positive samples were confirmed by virus isolation in embryonated chicken eggs.

SUMMARY

- Low pathogenic influenza infections can result in drastic declines in egg production in breeder turkeys.
- The viruses use avian type influenza receptors to infect the oviduct and replicate to high titers.
- Low pathogenic influenza viruses can be present in the eggs (albumin and egg shell surfaces) from infected turkeys.

CONCLUSIONS

Our study demonstrates for the first time that low pathogenic influenza viruses can be transmitted to internal egg contents. Caution should be enforced when raw eggs are used in food products that are consumed raw. Similarly, contamination of premises and hatcheries should be safeguarded against. Our experimental system will serve as a suitable model for research on egg associated dissemination of low pathogenic influenza viruses.

ACKNOWLEDGEMENTS

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